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1 ***Saonia flava* gen. nov., sp. nov., a marine bacterium of the family *Flavobacteriaceae***  
2 **isolated from coastal seawater.**

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10

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16

17 **Summary**

18 A Gram-negative, aerobic, yellow pigmented, straight rod, strain MOLA117<sup>T</sup>, was  
19 isolated from a coastal water sample from the north-western Mediterranean Sea, near  
20 Banyuls-sur-mer, France. On the basis of phylogenetic analysis of the 16S rRNA gene  
21 sequence, strain MOLA117<sup>T</sup> was placed within the family *Flavobacteriaceae*, but  
22 showed less than 93% 16S rRNA gene sequence similarity to other recognized species  
23 within the family. The most closely related genera included *Arenibacter*, *Cellulophaga*,  
24 *Maribacter* and *Zobellia*. The only isoprenoid quinone was MK6 and the fatty acid iso-  
25 C17:0 3-OH was dominant, representing over 33% of the total fatty acids. The DNA  
26 G+C content was 36.9 mol%. MOLA117<sup>T</sup> required NaCl for growth, did not exhibit  
27 gliding motility or produce flexirubin. Based upon the phenotypic and phylogenetic data,  
28 we propose that strain MOLA117<sup>T</sup> should be considered to represent a novel genus and  
29 species, for which the name *Saonia flava* gen. nov., sp. nov. is proposed. The type strain  
30 of *Saonia flava* is MOLA117<sup>T</sup> (=CIP 110873<sup>T</sup> = DSM 29762<sup>T</sup>). The accession number of  
31 the MOLA117<sup>T</sup> 16S rRNA gene sequence is AM990891.

32

33           The marine environment continues to be a major source of uncharacterized  
34 microorganisms with potentially interesting activities. These activities can be of  
35 academic interest but can also result in biotechnological applications. At the  
36 Oceanological Observatory in Banyuls, we aim to isolate prokaryotes that are  
37 environmentally ubiquitous in marine coastal ecosystems to study their biodiversity and  
38 function as well as to explore their use in biotechnology. Each isolated strain is added to  
39 the Banyuls Bacterial Culture Collection (WDCM911). During such an effort we isolated  
40 a Gram-negative, aerobic, yellow-pigmented bacterium, strain MOLA117<sup>T</sup>, from a water  
41 sample taken off the coast of Banyuls-sur-Mer, France. The closest cultured relatives,  
42 based on their 16S rRNA gene sequence, were species within the genus *Zobellia*, and the  
43 closest described species was *Zobellia uliginosa* strain ATCC 14397 with 92.7 % identity  
44 (BLAST [1]). Some species within the genera *Arenibacter*, *Cellulophaga* and *Maribacter*  
45 also showed some similarity (close to 92%) to MOLA117<sup>T</sup>. The highest identities to  
46 environmental sequences were 94%-93% to three 16S sequences retrieved from the gut of  
47 a wood feeding gastropod [2]. Thus, we suggest that MOLA117<sup>T</sup> represents a novel  
48 genus within the family *Flavobacteriaceae*. Our aim was to investigate the exact  
49 phylogenetic position and to phenotypically describe strain MOLA117<sup>T</sup>.

50           Strain MOLA117<sup>T</sup> was isolated from a seawater sample collected in February  
51 2004 from 3 m depth at station SOLA located in the bay of Banyuls-sur-Mer (42° 29' N  
52 3° 08' E). The isolation and bacterial stock procedures were performed as described in  
53 [3] using modified R2A agar (BD Co., Franklin Lakes, NJ) with 75% filtered  
54 Mediterranean seawater. Subsequent growth experiments were realized using Marine  
55 Agar (MA, BD Co.) or Marine Broth (MB, BD Co.) in the dark at 25°C. Cellular

56 morphology and size were determined using liquid culture incubated for 4 days, which  
57 corresponds to end of log phase/beginning of stationary phase (data not shown), and a  
58 Model H-7500 transmission electron microscope (Hitachi, Tokyo, Japan) as previously  
59 described [4], see figure S1.

60 Motility was tested using the hanging drop technique combined with light  
61 microscopy (model AX70 microscope, Olympus, Tokyo, Japan) with liquid cultures  
62 incubated for 4 days. Most of the tests for phenotypic characterization were performed as  
63 previously described [3]. Briefly, this included Gram staining using the Ryu KOH  
64 reaction [5], determination of oxidase activity using the oxydase reagent kit (bioMérieux,  
65 Marcy l'Etoile, France) and testing for catalase activity by determination of gas  
66 production upon exposure to a 3 % hydrogen peroxide solution. Formation of H<sub>2</sub>S was  
67 tested with Hydrogen Sulfide Test strips (Sigma Aldrich, St Louis, MO) with cultures  
68 grown on MB for 3 days. The requirement and tolerance of MOLA117<sup>T</sup> to NaCl was  
69 determined at 25 °C for 6 days on MB with NaCl concentrations ranging from 0-15 %, or  
70 more precisely 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 10, 13 and 15 %. To verify the optimal and  
71 tolerated pH levels for growth, MOLA117<sup>T</sup> was incubated in MB at 25 °C for 6 days  
72 amended with different buffers to stabilize the pH as follows: pH 4, 5 and 6 (MES), pH 7  
73 and 8 (HEPES) and pH 9 and 10 (AMPSO). Growth temperatures was determined on  
74 MA after 4 days of growth at temperatures ranging from 4 to 44 °C, or more accurately 4,  
75 10, 15, 20, 25, 30, 37 and 44°C. Antibiotic resistance was determined using antibiotic-  
76 impregnated paper discs on MA with a lawn of MOLA117<sup>T</sup> that was incubated at 25°C  
77 for 4 days. Production of flexirubin was evaluated by flooding a 4 day old plate with 20%  
78 (w/v) KOH (positive for flexirubin if color change from yellow to red, purple or brown).

79 Hydrolysis of starch, aesculin and Tween 80 was tested according to Lányi (1988) [6].  
80 Growth on different single carbon sources was verified with media supplemented with 1  
81 % of each carbon source [7]. The carbon sources tested included acetate, arabinose,  
82 cellobiose, citrate, dextran, galactose, glucose, glutamic acid, glycerol, formate, fumarate,  
83 lactate, lactose, maltose, pyruvate, succinate, sucrose, sorbitol, trehalose and xylose. To  
84 determine whether strain MOLA117<sup>T</sup> was able to degrade cellulose we utilized both filter  
85 paper dissolved in agar and filter paper in carbohydrate free broth [8], as well as CM-  
86 cellulose [9].

87 Additional carbohydrate metabolism was tested using the API 20NE and API  
88 50CH test systems (bioMérieux). Enzymatic activities were investigated using the API  
89 ZYM system (bioMérieux). For these tests, the medium utilized had the following  
90 composition: 25 g/L NaCl, 8g/L MgCl<sub>2</sub> and 0.5g/L KCl and the cultures were incubated  
91 at 30°C for a week in the dark [9].

92 Biomass for fatty acid analysis of MOLA117<sup>T</sup> was harvested from MA plates  
93 grown at 25°C for 4 days. Biomass for polar lipids and respiratory quinone analyses was  
94 harvested from cells grown on MB at 25°C for 4 days and cell pellets from 3 L of culture  
95 were lyophilized and sent for analysis. Analysis of fatty acid methyl esters, respiratory  
96 quinones, polar lipids and GC content was performed by the DSMZ Identification  
97 Service (DSMZ, Braunschweig, Germany) according to their protocols  
98 (<http://www.dsmz.de/services/services-microorganisms/identification.html>).

99 The sequence of the 16S rRNA gene of MOLA117<sup>T</sup> was determined from PCR  
100 products amplified after genomic DNA extraction with the Wizard Genomic DNA  
101 purification kit (Promega, Madison, WI) as previously described in Fagervold *et al.* [3].

102 Bacterial primers 27Fmod (AGRGTTTGATCMTGGCTCAG) [10][11] and 1492Rmod  
103 (TACGGYTACCTTGTTAYGACTT) [12] were used for PCR amplification. PCR  
104 products were purified using the Agencourt AMPureXP purification kit (Beckman  
105 Coulter France, Villepinte, France) and sequenced with an AB3130xl genetic analyzer  
106 (Life Technologies).

107 Sequence similarities of strain MOLA117<sup>T</sup> to sequences available in the NCBI  
108 GenBank were analyzed using the Basic Local Alignment Search Tool (BLAST)[1].  
109 Phylogenetic analysis was performed as previously described [3], using the ARB  
110 software package [13] and the RAxML Webserver (<http://www.phylo.org>) for bootstrap  
111 analysis (450 replicates) [14]. This analysis revealed that strain MOLA117<sup>T</sup> was  
112 phylogenetically related to species within the genera *Arenibacter* and *Cellulophaga*.  
113 However, MOLA117<sup>T</sup> clearly does not group with any of these genera since the bootstrap  
114 values clustering species within the genus *Arenibacter* together were high, as were the  
115 bootstrap values clustering species within the genus *Cellulophaga*. Nevertheless, the  
116 exact phylogenetic position of MOLA117<sup>T</sup> is uncertain since the bootstrap values for  
117 clustering MOLA117 with either of the genera *Arenibacter* or *Cellulophaga* are low (see  
118 Figure 1). Also, it is clear from the phylogenetic analysis that MOLA117<sup>T</sup> does not  
119 belong to any of the other described genera in the family *Flavobacteriaceae*.

120 Morphological, physiological and biochemical properties of strain MOLA117<sup>T</sup>  
121 are listed in Table 1 and in the genus and species descriptions. The major respiratory  
122 lipoquinone is MK-6, which is also the major respiratory quinone in *Flavobacteriaceae*  
123 [15].

124 Pigmentation, salinity requirement for growth and gliding motility are some  
125 important characteristics for the description of genera within the family  
126 *Flavobacteriaceae*. All the closest described genera and MOLA117<sup>T</sup> are pigmented, but  
127 only *Zobellia galactivorans* Dsij<sup>T</sup> [9] is positive for flexirubin. This strain also exhibits  
128 gliding motility, as do *Cellulophaga lytica* 23178<sup>T</sup> [16] and *Maribacter sedimenticola*  
129 KMM 3903<sup>T</sup> [17], but not MOLA117<sup>T</sup> and *Arenibacter latericius* KMM426<sup>T</sup> [18][19].  
130 Indeed, *Arenibacter latericius* KMM426<sup>T</sup> and MOLA117<sup>T</sup> are similar in many  
131 characteristics, but they differ in that MOLA117<sup>T</sup> can hydrolyze starch while *Arenibacter*  
132 *latericius* KMM426<sup>T</sup> cannot and that MOLA117<sup>T</sup> is susceptible to kanamycin. They also  
133 exhibit different ranges of growth temperature and pH tolerance; MOLA117<sup>T</sup> is the only  
134 one that is negative for growth at both 4°C, 37°C and 42°C. Further, even though all  
135 compared strains require NaCl for growth (Table 1), MOLA117<sup>T</sup> is slightly less  
136 halotolerant than related genera (Table 1), as indicated by the more narrow range of NaCl  
137 concentrations required for growth. MOLA117<sup>T</sup> is the only species, of those compared,  
138 which do not reduce nitrate.

139 The polar lipids that were extracted from MOLA117<sup>T</sup> could not be identified in  
140 detail by thin layer chromatography and subsequent staining (Figure S2). Acid production  
141 was observed with only a few substrates using single carbon sources with the API 50CH  
142 system and the API20NE test showed a relatively narrow range of carbon source  
143 utilization. However we did see a somewhat wider range when the carbon sources were  
144 tested with more specialized media and a 1% carbon source [7] meaning that the carbon  
145 utilization patterns from only API 50CH and the API20NE systems probably  
146 underestimate the range of substrates MOLA117<sup>T</sup> can utilize.

147 The most predominant fatty acid found in strain MOLA117<sup>T</sup> was iso-C<sub>17:0</sub> 3-OH  
148 at 33.21 % and this fatty acid is found in related genera, like *Zobellia*, *Cellulophaga* and  
149 *Maribacter* in relatively high amounts (from 14.9-20.8%), but was not found in the genus  
150 *Arenibacter*. Other major fatty acids found in MOLA117 were: C<sub>15:0</sub> (1.17 %), C<sub>17:1</sub> (1.41 %), iso-C<sub>15:0</sub> (11.44 %), iso-C<sub>15:1</sub> (7.12 %), anteiso-C<sub>15:0</sub> (1.26 %), iso-C<sub>17:1</sub> (2.62 %), C<sub>16:0</sub> 3-OH (2.08 %), iso-C<sub>15:0</sub> 3-OH (7.41%), summed feature 3 (4.14 %), and an  
153 unknown fatty acid at 14.90 %. Generally, all the genera we compared directly contained  
154 C<sub>15:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>15:1</sub> and anteiso-C<sub>15:0</sub> in varying amounts. The main difference  
155 between MOLA117<sup>T</sup> and the related genera with respect to fatty acids is that MOLA117<sup>T</sup>  
156 contains much more iso-C<sub>17:0</sub> 3-OH than strains in related genera.

157 In summary, strain MOLA117<sup>T</sup> is different from related genera in numerous  
158 metabolic capabilities listed in Table 1 and discussed above. Thus, both the phenotypic  
159 and phylogenetic data suggest that strain MOLA117<sup>T</sup> should be placed in a new genus  
160 *Saonia* gen. nov., with *Saonia flava* sp. nov. as the type species and MOLA117<sup>T</sup> as the  
161 type strain.

162

### 163 **Description of *Saonia* gen. nov.**

164 *Saonia* (Sa.o'ni.a L. fem. N. Sao, the nereid Sao; N.L. fem. n. *Saonia* named after  
165 Sao, the Nereid of "safe" passage, or the rescue of sailors, referring to the marine origin.)

166 Gram-negative, non-motile, aerobic and chemo-organoheterotrophic. Catalase,  
167 oxidase and alkaline phosphatase positive. Requires NaCl for growth. Produces yellow  
168 pigment but no flexirubin. The most predominant fatty acid is iso-C<sub>17:0</sub> 3-OH and the only  
169 respiratory lipoquinone is MK-6. Based upon the 16S rRNA gene sequence, the genus

170 forms a separate branch within the family *Flavobacteriaceae* close to the genera  
171 *Arenibacter* and *Cellulophaga*. The type species is *Saonia flava*.

172

173 **Description of *Saonia flava* sp. nov.**

174 *Saonia flava* (fla'va. L. fem. adj. *flava* yellow).

175 In addition to properties given in the genus description the species is characterized  
176 as follows. Cells are 1.5-3.4  $\mu\text{m}$  long and 0.5-0.6  $\mu\text{m}$  wide, non-spore forming, straight  
177 rods. The cells have an EPS like structure and they form aggregates in liquid culture.  
178 Colonies are yellow, smooth and slightly convex on MA. Growth occurs at temperatures  
179 between 15 and 30  $^{\circ}\text{C}$ . Optimal pH for growth is 7, and growth occurs at pH 7-8. Growth  
180 occurs in the presence of 1-4% NaCl, but the optimal NaCl concentration is 3 %.  $\text{H}_2\text{S}$  is  
181 not produced. Nitrate is not reduced under aerobic conditions. Using the API 20NE  
182 system, MOLA117<sup>T</sup> is shown to be positive for urease activity, aesculin hydrolysis and  
183  $\beta$ -galactosidase utilization and negative for denitrification, gelatin hydrolysis, indole  
184 production, D-glucose fermentation, arginine dihydrolase,  $\beta$ -glucosidase, and utilization  
185 of D-glucose, L-arabinose, D-mannose, D-mannitol, capric acid, N-acetylglucosamine,  
186 D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate and  
187 phenylacetic acid. Acid production was only observed with a few substrates using the  
188 API 50CH system, namely fructose, aesculin, saccharose, trehalose, inulin, raffinose and  
189 starch. MOLA117<sup>T</sup> could assimilate cellobiose, arabinose, maltose, trehalose, xylose,  
190 dextran, lactate, glycerol, sucrose, galactose, glucose and sorbitol as single carbon  
191 sources but not lactose, fumarate, succinate, formate, pyruvate, citrate, glutamic acid and  
192 acetate. When assayed with the API ZYM system, alkaline phosphatase, esterase lipase

193 (C8), leucine arylamidase, valine arylamidase, acid phosphatase and *N*-acetyl- $\beta$ -  
194 glucosaminidase are present, but esterase (C4), lipase (C14), cystine arylamidase, trypsin,  
195  $\alpha$ -chymotrypsin, naphthol-AS-BI-phosphohydrolase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -  
196 glucuronidase,  $\alpha$ -glucosidase,  $\beta$ -Glucosidase,  $\alpha$ -mannosidase and  $\alpha$ -fucosidase are  
197 absent. MOLA117<sup>T</sup> is susceptible to cefotaxime (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), kanamycin  
198 (30  $\mu$ g), vancomycin (30  $\mu$ g), rifampicillin (30  $\mu$ g), chloramphenicol (50  $\mu$ g), ampicillin  
199 (10  $\mu$ g), and resistant to erythromycin (15  $\mu$ g), penicillin (20 U), polymyxin (50  $\mu$ g),  
200 streptomycin (10  $\mu$ g), tetracycline (30  $\mu$ g). The DNA G+C content is 36.9 mol %.

201 The type strain MOLA117<sup>T</sup> (=CIP 110873<sup>T</sup> = DSM 29762<sup>T</sup>) was isolated from  
202 seawater from the northwestern Mediterranean Sea off the coast of Banyuls-sur-Mer,  
203 France.

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209

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215

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- 279

280 **Table 1**

<b>Characteristics</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
%ID 16S	-	93	91	90	90
Flexirubin	-	+	-	-	-
Gliding motility	-	+	-	+	+
Growth at 4°C	-	-	-	+	+
Growth at 37°C	-	+	+	+	-
Growth at 42°C	-	+	+	-	-
NaCl range for growth (%)	1-4	0.5-6	1-7	1-5	1-6
pH range for growth	7-8	6-8.5	6-10	7-9	5.5-10
Nitrate reduction (aerobic)	-	+	+	+	+
Hydrolysis of:					
Aesculin	+	+	ND	+	+
Agar	-	+	-	+	+
Gelatin	-	+	-	+	-
Starch	+	+	-	+	+
Tween 80	-	-	-	-	+
Urea	+	-	+	ND	-
Cellulose paper	-	-	-	ND	-
CM cellulose	-	-	-	+	-
DNA G+C content (mol%)	36.9	43	37.5	33	37.5
Antibiotics susceptibility:					
Kanamycin	+	-	-	-	-
Ampicillin	+	-	+	-	-
Penicillin	-	ND	-	-	-
Polymyxin	-	ND	-	-	-
Tetracycline	-	-	-	-	+

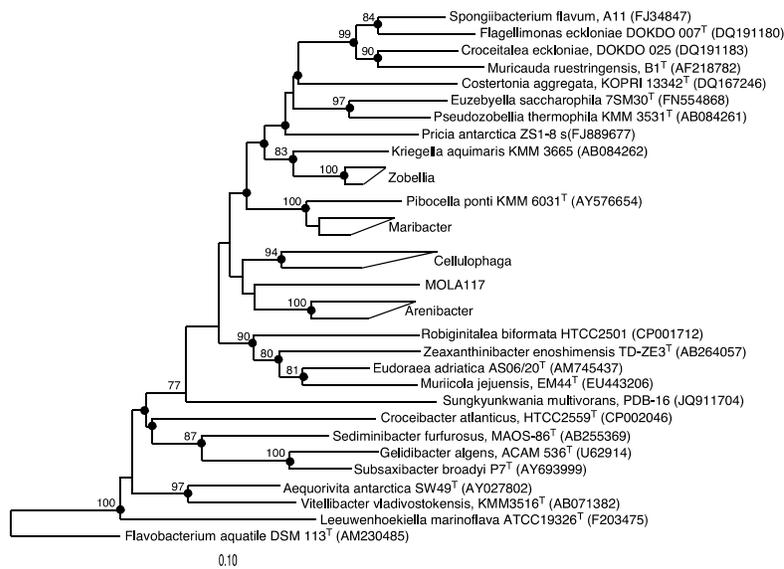
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283 **Table and figure legends.**284 **Table 1: Phenotypic characteristics of strain MOLA117<sup>T</sup> and type species of**285 **phylogenetically similar genera of the Flavobacteriaceae**286 Taxa: 1, MOLA117 (data from the present study); 2, *Zobellia galactivorans* Dsij<sup>T</sup> [9] ; 3,287 *Arenibacter latericius* KMM426<sup>T</sup> [18][19]; 4, *Cellulophaga lytica* 23178<sup>T</sup> [16], and [20] ;288 5, *Maribacter sedimenticola* KMM 3903<sup>T</sup> [17]. All are gram negative, aerobic rods with

289 yellow or yellow orange pigmentation, requiring NaCl for growth, growth at 25°C and

290 have MK-6 as the major respiratory lipoquinone. All were positive for catalase and  
 291 oxidase activity. All were negative for H<sub>2</sub>S production and resistant to streptomycin. +,  
 292 positive; -, negative; ND, no data available; v, variable  
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297 **Figure 1.** Maximum likelihood phylogenetic tree based upon 1180 positions of the 16S  
 298 rRNA gene sequence of MOLA117<sup>T</sup> and related *Flavobacteriaceae* available through the  
 299 NCBI GenBank database (accession numbers in parentheses). Bootstrap values greater  
 300 than 70% are given as percentages at branching points. Branching was also verified by  
 301 the neighbor joining method (black circles). Bar represents 0.10 substitutions per  
 302 nucleotide position. The groups on the tree are not formal groups. The group “Zobellia”  
 303 constitutes *Zobellia russellii* KMM 3677<sup>T</sup> (AB121976), *Zobellia amurkyensis* KMM  
 304 3526<sup>T</sup> (AB121974), *Zobellia laminariae* KMM 3676<sup>T</sup> (AB121975), *Zobellia*  
 305 *galactivorans* Dsij<sup>T</sup> (AF208293) and *Zobellia uliginosa* (M62799). The group

306 “Maribacter” constitutes *Maribacter antarctius* CL\_AP4<sup>T</sup> (EU512921), *Maribacter*  
307 *acticus* KOPRI 20294<sup>T</sup> (AY771762), *Maribacter orientalis* KMM 3947<sup>T</sup> (AY271624),  
308 *Maribacter aquivivus* KMM 3949<sup>T</sup> (AY271625), *Maribacter stanieri* KMM6046<sup>T</sup>  
309 (EU246691), *M. dokdonensis* DSW-8<sup>T</sup> (AY960749), *Maribacter sedimenticola*  
310 KMM03903<sup>T</sup> (AY271623), *Maribacter forsetii* KT02ds18-6<sup>T</sup> (AM712900) and  
311 *Maribacter ulvicola* KMM3951<sup>T</sup> (AY271626). The group “Cellulophaga” includes  
312 *Cellulophaga algicola* IC166<sup>T</sup> (AF0001366), *Cellulophaga baltica* NN015840  
313 (AJ005972), *Cellulophaga pacifica* KMM3664<sup>T</sup> (AB100840), *Cellulophaga*  
314 *tyrosinoxydans* EM41<sup>T</sup> (EU443205), *Cellulophaga fucicola* NN015860 (AJ005973),  
315 *Cellulophaga lytica* DSM7489<sup>T</sup> (CP002534) and *Cellulophaga geojensis* M-M6<sup>T</sup>  
316 (HQ596527). The group “Arenibacter” includes *Arenibacter certesii* KMM 3941<sup>T</sup>  
317 (AY271622), *Arenibacter latericius* KMM 426<sup>T</sup> AF052742, *Arenibacter nanhaiticus*  
318 NH36A<sup>T</sup> (EU999955), *Arenibacter echinorum* KMM 6032<sup>T</sup> (EF536748), *Arenibacter*  
319 *palladensis* LMG 219<sup>T</sup> (AJ575643) and *Arenibacter troitsensis* KMM3674<sup>T</sup>  
320 (AB080771).